Ameliorative Effect of Water Treatment Residual Nanoparticles on Seed Germination of Cucumber (*Cucumis sativus* L.) under Cd Stress

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ABSTRACT

The effects of water treatment residual nanoparticles (nWTRs) on germination performance of cucumber seeds exposed to Cd stress were investigated. The results indicated that germination percentages of unprimed seeds which exposed to Cd stress of 10 and 100 (mg/l) decreased by 25% compared to control. Seed priming with nWTRs nanoparticles significantly improved germination performance under Cd stress. The highest germination percentage (%) was observed with the nWTRs treatment at concentrations of 250mg/l and higher. The percentage of root length enhancement as a result of nanoparticles applications at rates of 250, 500, 1000 mgl^{-1} were 215.62 %, 446.87% and 479.7% respectively. Thus, priming with WTR nanoparticles at concentrations higher than 200mgl^{-1} may have significant beneficial effects on germination performance of Cd stressed cucumber seeds.

Keywords: Seed treatment, Germination rate, Root length, Root radius.

INTRODUCTION

Stressful environment is considered a potential agricultural threat for the sustainable agriculture. Stressful environment may adversely affect plants physiological and metabolic processes, and finally diminish growth development and productivity. More than 50% of crop yield reduction worldwide is due to abiotic stress (Hopkins and Hüner, 2009).

Seed priming is known as the seed treatment which improves seed performance under environmental condition. It is reported that seed priming is a useful and recommended practice to enhance rapid and uniform emergence for the purpose of achieving high germination rate, high seedling vigor and better yields in vegetables,and floriculture and some field crops (Parera and Cantliffe, 1994; Toklu et al., 2015). Ghassemi-Golezani et al. (2008) reported that hydro priming is an eco- friendly simple, and low cost technique, for improving seed germination and seedling vigor of lentils. Kaya et al. (2006) observed significant increase in germination and seedling growth due to hydropriming under salt and drought stresses. Lemrasky and Hosseini (2012) indicated that the greatest stem and radicle length of wheat seedlings were obtained after seed priming with 2% KCl and 4% KCl for 45 h.

Recently, Cadmium, is known to be the most hazardous pollutant in environment and has been included on the USEPA priority pollutants list (Nedelkoska and Doran, 2000). The Cd stress effects and mechanisms on plants have been widely studied (Nedelkoska and Doran, 2000). However, few researches have reported the effect of nanoparticls on reduction of Cd phytotoxicity in environmental mediums. A short-term study was conducted by Wang et al., (2012) using six nanoparticles materials ((Kaolin, montmorillonite, hydroxyapatite, Fe_3O_4, α-Fe_2O_3 and γ -Fe2O3) to determine the impact of nanoparticles on the toxicity of Cd to the root growth of different plants species after Cd exposure. They found that the root growth was not significantly inhibited by the presence of NPs except for hydroxyapatite on tomato.

Water treatment residuals (WTRs) are by-product of water purification industry. Because of the unique properties of WTR nanoparticles (nWTRs), one may hypothesize that its application in agricultural soils would substantially increase metal adsorption on amorphous nanoparticles and mitigate the adverse effect of toxic metal accumulation in crops. Information are not available, however on the role of WTR nanoparticles on mitigating the phytotoxicity of Cd to cucumber plants. Therefore the objectives of this study were to investigate and validate the effects of different concentrations of nWTRs suspensions in the presence of two concentrations of Cd ions on cucumber seed germination properties and seedling growth.

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MATERIALS AND METHODS

1. Water treatment residuals (WTRs) and cucumber seeds

The WTRs were obtained from a local drinking water treatment facility in Alexandria, Egypt .The WTRs samples were collected transported to the laboratory and air-dried. Seeds of cucumber (Cucumis sativus) were obtained from Egyptian ministry of agriculture and refrigerated (4 C^o) until use.

2. Production and characterization of WTR nanoparticles (nWTRs)

The general physiochemical properties of the WTRs were determined according to standard methods (Tan, 1996). The chemical properties of WTRs were determined as follows: the pH was measured in 1:2.5 suspensions (WTRs: deionized water ratio of 1:2.5) using pH meter. The general physicochemical properties of the WTRs are shown in Table1. Sub-samples of WTRs were ground and passed through sieves having 51µm of pore diameter. Nanoparticls of WTR (nWTRs) were produced following the method of Elkhatib et al. (2015). The surface morphology, composition and element contents of the WTRs nanoparticles were investigated using scanning electron microscopy (SEM) with energy dispersive X-ray (INCAx-Sight model 6587, Oxford Instruments, UK). The specific surface area of nWTRs was determined according to Brunauer–Emmett –Teller method (Brunauer et al., 1938) by using surface area analyzer (Quantachrome Instruments, USA).

Table 1. The general physicochemical properties of the WTRs

Characteristics	Units	WTRs
pH		7.45 ± 0.06
EC	dSm^{-1}	1.67 ± 0.04
O.M†	g kg^{-1}	57.00 ± 2.00
KCl-Al	mg kg^{-1}	28.18 ± 1.03
Olsen-P	mg kg^{-1}	24.00 ± 2.00
CEC	Cmol(+) kg^{-1}	34.78 ± 0.34
DTPA-Ni	mg kg^{-1}	2.49 ± 0.07
DTPA Pb	mg kg^{-1}	1.58 ± 0.04
DTPA Cd	mg kg^{-1}	0.09 ± 0.02

pH was measured in sample/water suspension (1:2.5); EC: electrical conductivity; O.M: organic matter; CEC: cations exchange capacity.

3. Exposure suspensions and Cd solutions

Suspensions of water treatment residuals nanoparticles (nWTRs) were daily prepared in concentrations of 0 (control), 10, 100, 250, 500, 750 and 1000 mgL^{-1}in deionized water (DI-water). The pH of each suspension was adjusted to 6.8+ 0.2 by 0.1NHCl and/or NaOH before plant exposure. Cadmium solutions in concentrations of 10 and 100 mgL^{-1} were also prepared using Cd Cl_2 and DI-water. Chemicals of analytical grade including Cd Cl_2 were obtained from Sigma Chemical. Co. (St Louis, MO, USA).

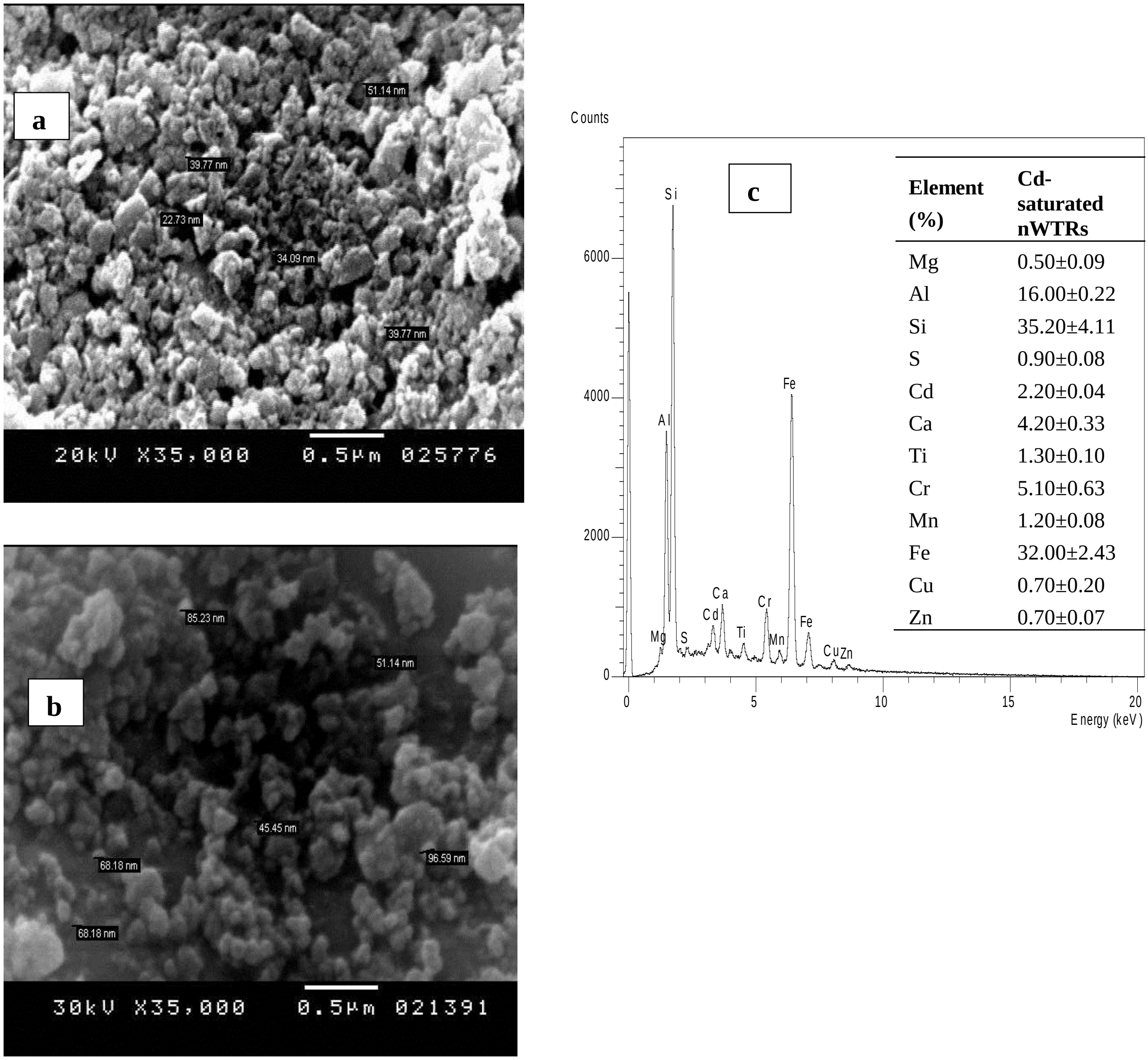
4. Seed germination trial

Uniform size cucumber seeds were sterilized using 3% H_2O_2 solution and thoroughly washed with distilled water prior to use. The sterilized seeds were immersed in Erlenmeyer flasks containing nWTRs suspensions with concentrations of 0 (control), 10, 100, 250, 500, 750 and 1000 mg/l in DI water for 3 h, and then germinated on moist filter paper in Petri dishes at 28ºC. Five seeds were placed on each Petri dish and three replicates were used for each nWTRs concentration. All dishes were placed in a growth chamber under dark conditions at 26ºC for germination using complete randomized design. The DI water was added to the petri dishes daily for 7 days. Germination rate was recorded after 36 h and seedling roots lengths were measured after 7days using a millimeter ruler. Seedling viability and growth as affected by exposure to WTR nanoparticles and Cd solutions were also determined.

RESULTS AND DISCUSSION

1. Characterization of Water Treatment Residual Nanoparticles (nWTRs)

Production of nanoscale WTR (nWTRs) was achieved by milling subsamples of nWTR (<51um) (Elkhatib etal. 2015). Scanning electron microscopy (SEM) with energy dispersive X-ray (INCAx-Sight model 6587, Oxford Instruments, UK) were used to characterize nWTR (Elkhatib etal. 2015a). The microstructure of WTR nanoparticles as examined by SEM indicated the spherical shape of WTR nanoparticles. The representative single particle sizes are typically less than 100 nm in diameter (Fig.1a). The major elements in nWTR as SEM-EDX indicated are Si, Al, Fe, and they represent around 83 % of the total elements (Fig.1a). The X-ray diffraction analysis (Elkhatib etal. 2015b) suggested that amorphous iron, aluminum (hydr) oxides and silicon oxide dominated all nWTR, with no apparent crystalline iron–Al (hydr) oxides. The SEM image of Cd loaded nWTR (Fig.1 b) shows formation of a coating layer on the surface of the nWTR which indicates the reaction occurred on nWTR surface.



Element (%)	Cd-saturated nWTRs
Mg	0.50±0.09
Al	16.00±0.22
Si	35.20±4.11
S	0.90±0.08
Cd	2.20±0.04
Ca	4.20±0.33
Ti	1.30±0.10
Cr	5.10±0.63
Mn	1.20±0.08
Fe	32.00±2.43
Cu	0.70±0.20
Zn	0.70±0.07

FIg. 1. Scanning electron microscopy (SEM) image spectrum of (a) nWTR (b) the Cd-loaded nWTR and (C) energy dispersive X-ray (EDX) of Cd-loaded nWTR.

In addition, the SEM–EDX analysis spectrum (Fig.1b) ascertained the appearance of a cadmium peak (2.20 %) amongst the elements detected in Cd-loaded nWTR. The BET specific surface area (SSA) of nWTR, sample revealed that SSA of nanoscale WTR (129.0 m^2g^{-1}) is 2-3 times higher than SSA of nWTR (53.1 m^2g^{-1}) which demonstrates the high reactivity of nWTR and makes it ideal candidate for water treatment and desalination.

Effect of Priming with nWTRs on Cucumber Seeds Germination

i. Germination performance

The cucumber was selected because it is one of plant species recommended by USEPA (USEPA, 1996) for plant toxicity studies. The effects of WTR nanoparticles on germination performance of cucumber seeds exposed to Cd stress are shown in Table 2.

Table 2. Effect of WTR nanoparticles on germination of cucumber seeds exposed to Cd Stress

Cd conc. (mg/l)	Germination rate%						
	nWTR conc. (mg/l)						
	0	10	100	250	500	750	1000
0	80	80	80	100	100	100	100
10	60	80	80	100	100	100	100
100	60	80	80	100	100	100	100

Results indicated that germination percentages (%) of cucumber seeds were greatly affected when seeds exposed to Cd concentrations of 10 and 100mg/l. Germination percentages of unprimed seeds which exposed to Cd stress of 10 and 100(mg/l) decreased by 25% compared to control. Seed priming with WTR nanoparticles showed faster germination than unprimed seeds. Seeds primed for 36h resulted in earlier emergence than control (without any treatment) (Table 2). Seed priming significantly improved germination performance under Cd stress. The highest germination percentage (%) was observed with the nWTR treatment at concentrations of 250mg/l and higher. Thus, priming with WTR nanoparticles at concentrations higher than 200mgl^{-1} may have significant beneficial effects on germination performance of Cd stressed cucumber seeds.

ii. Root length (RL), root radius (RR) and root surface area (RSA) of cucumber seedlings

Cadmium stress and priming with WTRs nanoparticles had significant effects on root length (cm), root radius (mm) and root surface area (cm^2) of cucumber seedlings. The deleterious effect of Cd addition on root length of cucumber seedlings is clear as the mean of root length , root radius and root surface area were significantly affected by the Cd concentration added (Fig2). Significantly lower seedlings length of 35.79% and 88.77% were recorded in seeds that were treated with Cd concentration of 10and 100mgl^{-I} respectively. In contrary, root radius (0.446 mm) and root surface area(2.801cm^2) in the seedlings treated with 100mgl^{-1} were noticeably higher than control (0.149 mm and 0.938cm^2) respectively. Similar to our findings, Lux et al. (2011) reprted that decrease of root number, length and dry mass and enlargement of the root diameter are considered syndromes of Cd toxicity on roots. Lunáčková et al. (2003) indicated that roots of *Populus x euramericana* became shorter and thicker when treated with Cd. Similarly, increased root diameter of maize seedlings after Cd application was also observed (Maksimović et al. , 2007) .

Fig. (2) shows the root length of cucumber seedlings as affected by seeds priming with different concentrations (0, 10, 100, 250, 500 and 1000 mgl^{-1}) of WTR nanoparticles in the presence and absence of Cd ions. Exposure of cucumber seeds to WTR nanoparticles significantly promoted the root length of cucumber seedlings at concentrations from 250 mg/L to 1000 mg/L. The Enhancement rate (%) of the root seedlings length was determined by the following formula given by Chou and Lin (1976):

Enhancement rate (%) of Root = Root length of control (0 nWTR) - Root length of treatment/Root length of control X100

The effect of seed priming with different concentrations of nWTR on root length enhancement is shown in Table 3. It is clear that seed priming with WTR nanoparticles counter act Cd stress and significantly improved seedling root length at the two Cd levels (figs. 3 & 4). The percentage of root length enhancement as a result of nanoparticles applications at rates of 250, 500, 1000 mgl^{-1} were 215.62 %, 446.87% and 479.7% respectively. Wang etal. 2012 described that Multiwalled carbon nanotubes permeate tomato seeds and boost the germination rate by improving the seed water uptake.

Table 3. Effect of seed priming with different concentration of nWTR on root length enhancement.

nWTR (mg/l)	Root Length Enhancement(%)	
	Cd concentrations (mg/l)	
	10	100
10	1.9	81.25
100	8.20	112.5
250	12.30	215.62
500	31.15	446.87
750	33.33	468.73
1000	43.72	479.7

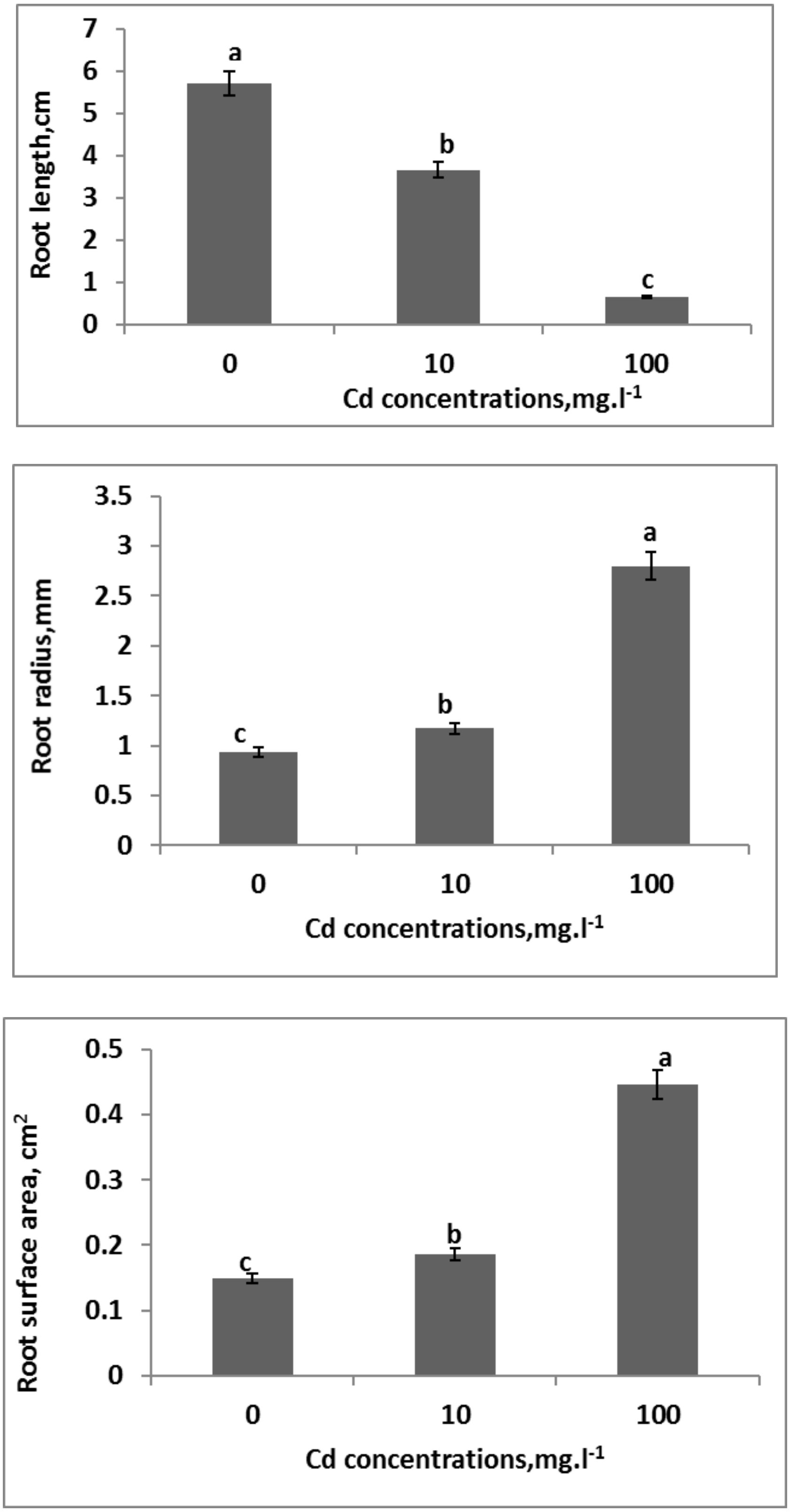


Fig 2. Effect of different Cd concentrations on root length, root radius and root surface area.

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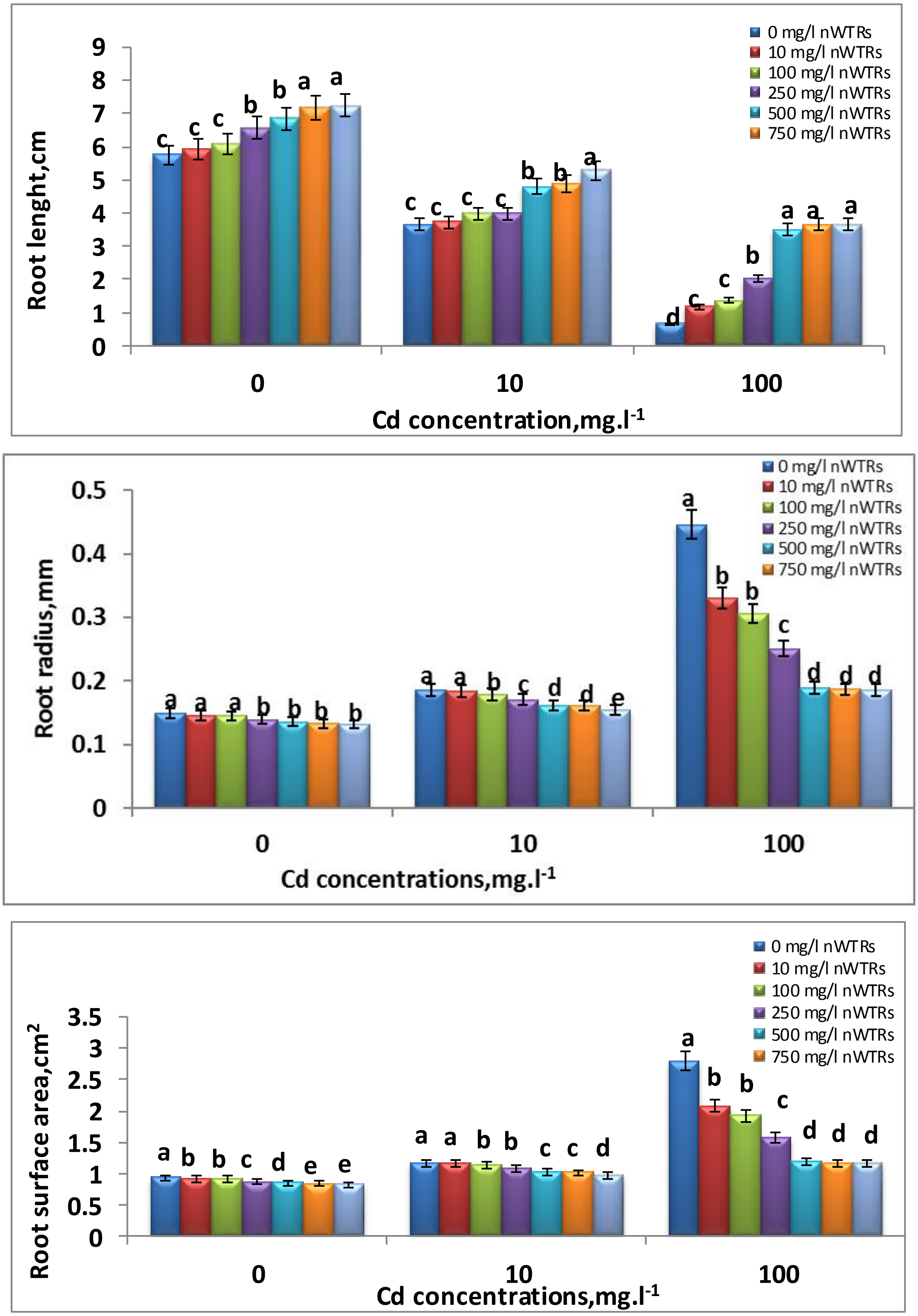

Fig 3. Effect of seed priming with different concentrations (0, 10, 100, 250, 500 and 1000mgl^{-1}) of WTR nanoparticles on root length (RL) root radius (RR) and root surface area (RSA) under Cd stress.

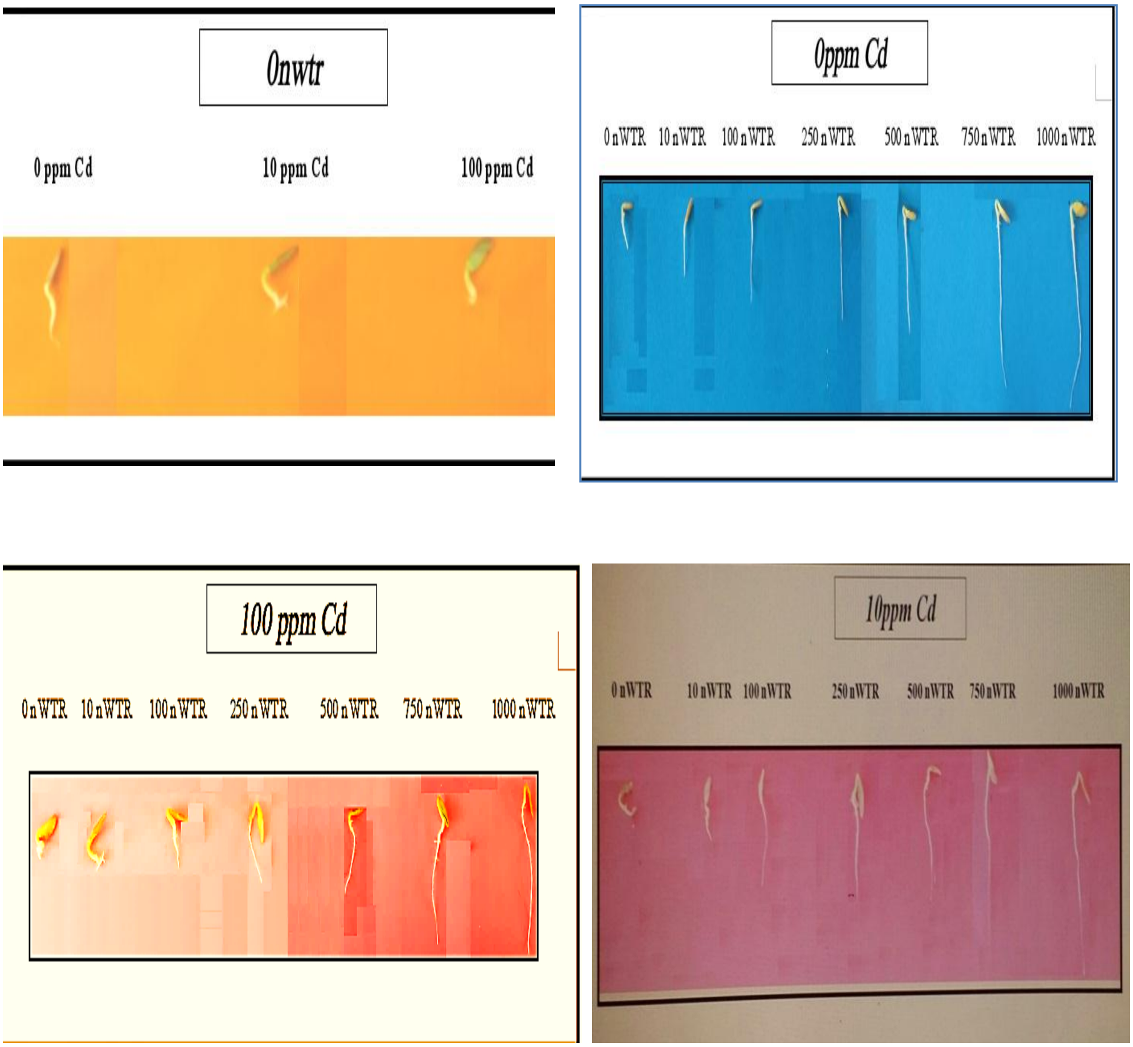

Fig. 4. Morphological picture of germinated seeds primed with nanoparticles or not primed under Cd stress.

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